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The effect of body weight changes and endurance training on 24 h substrate oxidation

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OBJECTIVE: To investigate the effect of exercise training and dietary macronutrient composition on 24 h substrate oxidation in male, obese subjects.

DESIGN: A 16 month exercise intervention study was executed, including a weight loss period with a very low energy diet (VLED) for 2 months at the start of the study.

SUBJECTS: Twelve male, obese subjects (age 36.3 ± 5.1 y; body weight 94.6 ± 13.9 kg; body mass index, BMI 30.8 ± 3.0 kg/m²) and in an additional study 15 lean, well-trained subjects (age 36.2 ± 7.2 y; body weight 72.2 ± 5.9 kg; BMI 22.3 ± 1.7 kg/m²) participated.

MEASUREMENTS: Substrate oxidation was measured during a standardized 36 h stay in the respiration chamber at the start of the study (0 months), and at 4, 10 and 16 months. In the respiration chamber subjects were randomly assigned to a high-fat (Hi.F) diet (60% of energy (En%) fat) or a reduced-fat (Red.F) diet (30 En% fat). The well-trained group was measured once in the respiration chamber for 36 h according to the same protocol.

RESULTS: At any time point, independent of the diet consumed, the 24 h carbohydrate (CHO) balances in the chamber were mostly negative (means ranging from +31 to -98 g/d) and the fat balances mostly positive (means ranging from -26 to +38 g/d) for the obese as well as for the lean, well-trained group. For both diets an increased shortage of 70 g of CHO was found at 16 months compared with 4 months, and an increase in fat balance of 33 g during the same time period in the obese subjects, indicating that CHO oxidation had increased with 12 months endurance training. In the well-trained group the 24 h CHO balance was even more negative for both types of diet (-103 to -185 g/d for the Red.F and Hi.F diet, respectively) under similar conditions compared with the trained obese group.

CONCLUSION: The changes in 24 h substrate utilization in the obese, as well as in the well-trained group, suggest that endurance training increased the reliance on carbohydrate oxidation and therefore did not increase 24 fat oxidation.

Keywords: obesity; substrate utilization; energy balance; training status

Introduction

Maintenance of body weight (BW) is achieved when energy intake and energy expenditure are balanced via manipulation of the type and quantity of food consumed or level of physical activity.¹ Over the last decade a number of studies have suggested that a positive energy balance, causing obesity, is mainly a result of a positive lipid balance,^{2–5} caused by decreased physical activity and an increased consumption of high-fat diets.^{6–8} To accomplish fat balance, oxidation of fat should be stimulated (by exercise or drugs) and/or fat intake should be decreased (low-fat diet).^{9–11} In normal-weight subjects, exercise training has been found to result in increased fat oxidation during rest and exercise.^{12–16} In contrast to normal-weight subjects, obese subjects were found to have a relatively impaired fat oxidation.¹⁷ Consumption of a high-fat diet will therefore easily result in a positive fat balance, and eventually an increase in body weight.^{5,18} Obese subjects are

therefore advised to eat a low-fat diet and perform endurance-type exercise to reduce the positive fat balance and prevent weight gain.^{19,20} The increased capacity to oxidize fat, as found in normal-weight subjects after training intervention,^{12–16} could be a useful mechanism for maintenance of body weight for obese subjects after a weight reduction period. However, does exercise training affect fat oxidation in the same way in weight-reduced obese subjects as in lean subjects?

To answer this question we undertook the following study. After a 4 month period of weight reduction and weight stabilization by means of a very low energy diet (VLED) and a physical activity program in obese men, a 12 month exercise intervention study was executed to investigate changes in 24 h substrate oxidation at different time points. The substrate balances were examined by means of 36 h measurements in a respiration chamber. It was further hypothesized that stimulation of fat oxidation in weight-reduced obese subjects caused by the exercise intervention would improve the fat balance even when acutely a high-fat diet is consumed. We therefore studied the effect of a high-fat diet (Hi.F diet; 60% of energy (En%) fat) and a reduced-fat diet (Red.F; 30 En% fat) on substrate oxidation. To compare these effects with a maximal effect of endurance training on substrate

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oxidation, the results of the obese group were compared with results of a lean, well-trained group of athletes following the same protocol in the respiration chamber. Although there are differences in subject characteristics, this comparison might give an indication of what might be achieved in the post-obese subjects with endurance training.

Methods

Subjects

Fifteen Caucasian, obese men, recruited via the local newspaper, participated in this study after a physical, medical examination (age 37.3 ± 5.2 y; BW 96.5 ± 13.6 kg; body mass index, BMI 30.9 ± 2.8 kg/m²). The very low energy diet (VLED) was started with 16 male obese subjects. One subject was not able to keep to the strict diet regime. Halfway into the study, three subjects were not able to continue the training program. Therefore data from 12 obese subjects are presented (age 36.3 ± 5.1 ; BW 94.6 ± 13.9 kg; BMI 30.8 ± 3.0 kg/m). For comparison of the 24 h substrate oxidation data during the standardized respiration chamber protocol, we also measured 15 very well trained athletes (age 36.2 ± 7.2 y; BW 72.2 ± 5.9 kg; BMI 22.3 ± 1.7 kg/m²), matched for age. On average, the well-trained athletes trained for 12.1 ± 7.4 h a week, and had a training history of 19.6 ± 7.7 y. All trained athletes participated in cycling or triathlon competitions. Written informed consent was obtained from each subject at the start of the study. The study protocol was reviewed and approved by the Medical Committee of Maastricht University.

Study design

The obese subjects kept to a VLED (Modifast[®], Novartis Nutrition, Switzerland) for 2 months in order to lose weight. Total energy intake during VLED was 2 MJ daily. After the VLED subjects were advised about a healthy, weight maintenance diet, but not restricted in their energy intake, and could eat *ad libitum*. All subjects took part in the endurance training program during the first 4 months of the study, of which the first 2 months were combined with the VLED. Intensity and performance time of the training were gradually built up because all subjects were inactive at the start of the study. After 1 month training the subjects ran and cycled at a moderate intensity (around 50% $\dot{V}O_{2\max}$ for at least 1 h, three to four times a week, and were coached by the investigator (WP). Exercise intensity was regularly controlled by heart rate monitoring. This training intervention was performed by all subjects to prevent a fast rebound of body weight after the VLED, and to have a similar starting point to study substrate utilization at 4 months. After 4 months the training sessions

were continued at a triathlon club where they were able to swim, cycle and run in group sessions (three to four times a week), supervised by a coach. Training intensity was continuously checked by monitoring the heart rate of two different subjects each training session and kept at 60–65% $\dot{V}O_{2\max}$. Therefore, absolute training output was adapted based upon the maximal oxygen uptake.

Compliance with endurance training during the weight maintenance phase

Compliance with training sessions was checked by a training diary (including day-to-day activities and remarks like illness, injury, weather conditions, etc), questionnaire (filled in at the laboratory when measurements took place) to check frequency of training (number of training hours a week) and by the investigator (WP) visiting training sessions to examine compliance from 4 to 16 months. Of the 15 participating subjects, three dropped out of the training program during the 12 month follow-up, five subjects trained once to twice a week, and seven subjects were able to keep the training frequency at a high level, three times a week or more.

Physical fitness

One week before the respiration chamber experiments, subjects performed a maximal performance test (an incremental exercise test) on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands) to estimate maximal power output (W_{\max}). After a warm-up period of 9 min (5 min at 40 W and 4 min at 80 W) the workload was increased every minute by 20 W until the work load could no longer be sustained. The maximal power output was calculated using the total time cycled in the exercise test. The highest workload completed for 1 min ($W_{\text{completed}}$) and the number of seconds (X) that the final increase of 20 W was maintained were added, according to the following formula: $W_{\max} = W_{\text{completed}} + ((X/60) \times 20)$. Criteria for maximal performance were forced ventilation, levelling off of oxygen uptake or a respiratory quotient (RQ) above 1.1. The oxygen uptake during the test was measured continuously, using a computerized open system (Oxycon Beta, Mijnhardt, Bunnik, The Netherlands).

Body composition

The deuterium dilution technique was used for measurement of body composition in this study.²¹ $^2\text{H}_2\text{O}$ dilution was used to measure total body water (TBW). Subjects were asked to collect a urine sample in the evening just before drinking the deuterium enriched water solution. After consumption of this solution no further consumption was allowed. Ten hours after drinking the water solution another urine sample was collected. The dilution of the deuterium isotope is a measure of total body water of the subject.²²

Deuterium was measured in the urine samples with an isotope ratio mass spectrometer (VG-Isogas Aqua Sira). TBW was obtained by dividing the measured deuterium dilution space by 1.04.²¹ Fat-free mass was calculated by dividing the TBW by the hydration factor 0.73. By subtracting FFM from BW, fat mass (FM) was obtained. FM expressed as a percentage of BW revealed body fat percentage (BF%).

Energy intake

The amount of food consumed was recorded 3 days prior to the respiration chamber measurement in a food intake diary. Subjects were asked to write down everything that was consumed (meals, drinks and snacks) before each respiration chamber experiment. After returning the diaries, estimation of the portion sizes was confirmed by examining household attributes to improve the estimation of the amount of food consumed. The diaries were analysed with the Dutch food table and the accessory computer program.²³

Respiration chamber experiment

At four fixed time points during the study (at month 0 and at 4, 10 and 16 months), subjects stayed in the respiration chamber for 36 h. Because of the weight loss of 13.7 ± 3.0 kg after 8 weeks VLED (range 8.2–18.5 kg), no respiration chamber measurement was performed at 2 months, since the influence of the weight loss would overrule the effect of exercise training.

Subjects came to the laboratory at 17.15 h and the respiration chamber measurement started at 18.00 h. The participating men were randomly assigned to a high-fat diet (Hi.F) or a reduced-fat diet (Red.F) in the respiration chamber. Energy intake (EI) was carefully adjusted to the estimated energy expenditure (EE), in order to achieve 24 h energy balance (EB). On the first evening, the dinner provided 40% of the energy needed to maintain the subjects' EB, based on resting metabolic rate (RMR) calculated from the equations of Harris and Benedict²⁴ multiplied by a physical activity factor of 1.5 (for the obese, inactive group) or 1.7 (for the well-trained group) for free living conditions. This difference in physical activity factor was based upon the differences in daily physical activity and exercise training frequency. On the first day of the test no physical training activities were allowed. After the first night the sleeping metabolic rate (SMR) was measured and, based upon this value and the calculated amount of planned work to be executed that day in the respiration chamber, the subjects were fed in order to achieve EB.

Respiration chamber conditions

Energy expenditure (EE) and respiratory quotient (RQ) were calculated from oxygen consumption and carbon dioxide production as measured in a respira-

tion chamber,²⁵ according to the formulae of Weir,²⁶ from 0.00 to 0.00 h the next day. The assumption was made that protein intake and oxidation were equal under these conditions in the respiration chamber, as previously observed in our laboratory.^{25,27,28} Oxidation of carbohydrate (CHO) and fat was calculated using the equations of Brouwer:²⁹

$$\text{Fat oxidation (g/d)} = 1.718 * \dot{V}O_2 - 1.718 * \dot{V}CO_2 - 0.315 * P$$

$$\text{CHO oxidation (g/d)} = 4.17 * \dot{V}CO_2 - 2.695 * \dot{V}O_2 - 0.390 * P$$

where $\dot{V}O_2$ is the oxygen consumption (L/d), $\dot{V}CO_2$ is the carbon dioxide production (L/d), and P is the protein oxidation (g/d).

Results of alcohol combustion tests (50–350 ml CO_2) showed an accuracy of $0.5 \pm 2.0\%$ for O_2 consumption and $-0.3 \pm 1.6\%$ for CO_2 production for 2–24 h experiments.²⁵ The physical activity of the subjects in the chamber was monitored by means of a radar system, based on the Doppler principle (Advisor DU 160, USA).

High-fat and reduced-fat diets

In the respiration chamber two groups, consuming either a high-fat (Hi.F) diet or a reduced-fat (Red.F) diet on each occasion, were studied. Exchange of macronutrients was carried out for CHO and fat. The Hi.F-diet contained 15 En% of protein, 60 En% of fat and 25 En% of CHO. The amount of fibre was 0.8 g/p MJ and the P/S ratio was 0.17. The Red.F-diet contained 15 En% of protein, 30 En% of fat and 55 En% of CHO. The amount of fibre per MJ was 1.2 g and the P/S ratio 0.46.

The meals were served at 8.00 h (breakfast); at 13.00 h (lunch) and at 18.00 h (dinner). The composition of the macronutrients for each meal was similar to the composition of the diet over the whole day. Breakfast contained 25 of the total energy intake (EI), lunch contained 35%, and dinner comprised 40% of the total EI. The meals were prepared with common food items like bread, cheese, jelly, sausage, milk, pasta, etc. Each food item was weighed to the nearest gram on a precise balance. In between the main meals subjects were allowed to drink water, tea or coffee without sugar and milk (only sweeteners were allowed), to prevent energy intake between the main meals.

EB was determined by subtracting 24 h EE from energy intake (EI). Macronutrient composition and total EI were calculated using the Dutch food composition table.²³ The food quotient (FQ), defined as the ratio of CO_2 produced to O_2 consumed during the

oxidation of a representative sample of the diet,³⁰ was calculated using the following equations:³¹

$$\begin{aligned} \text{O}_2 \text{ consumption (L/d)} = & (0.966 * \text{protein intake}) \\ & + (2.019 * \text{fat intake}) \\ & + (0.829 * \text{CHO intake}) \end{aligned}$$

$$\begin{aligned} \text{CO}_2 \text{ production (L/d)} = & (0.77 * \text{protein intake}) \\ & + (1.427 * \text{fat intake}) \\ & + (0.829 * \text{CHO intake}) \end{aligned}$$

where the intake of protein, fat and CHO is expressed in grams per day. For energy intake calculations, the differences in digestibility of the macronutrients, the Atwater factors, were taken into account (for protein, fat and CHO the factors are 0.909, 0.948 and 0.953 respectively).

Activity protocol

In the respiration chamber a strict activity protocol was kept. Subjects were woken at 7.15 h and were asked to sleep at 23.00 h. During their stay in the respiration chamber subjects were asked to cycle for 1 h at 40% of their \dot{W}_{\max} twice (at 10.00 and at 15.00 h). Efficiency of cycling of the subjects was assumed to be 21% and they cycled for 5 min at a fixed work load of 60 W during the warming-up and cooling-down. During the daytime subjects were allowed to move freely, to sit, lie down, telephone, listen to the radio and watch television; however sleeping and other exercise activities than those prescribed were not allowed.

Due to the training program the individual maximal aerobic capacity and power output increased during the course of the study. Therefore the relative work load cycled in the respiration chamber changed.

From the results at month 0 it became clear that energy balance was not reached. Therefore it was decided to ask the subjects to stay an additional 24 h in the chamber at 16 months (in total 60 h) to mimic the negative energy balance on an individual basis as much as possible. It also gave us the opportunity to compare 24 h substrate oxidation at the same initial (month 0) absolute work load. At the first day the normal procedure was performed (feeding subjects in energy balance and cycling at a relative work load of 40% \dot{W}_{\max}). During the second day exactly the same conditions regarding energy balance and absolute workload were imposed as measured during the first chamber measurement at month 0. This second day stay enabled us to compare substrate utilization changes caused by training and weight loss at the same absolute work load. Nine subjects were willing to participate in this part of the study.

Data analysis

Four major questions with respect to 24 h energy and substrate balances were studied:

1. Long-term training effect and the effect of body weight gain. The effect of training in the obese group was examined using an exercise protocol in the respiration chamber with a relative work load of 40% \dot{W}_{\max} . Comparison of the starting condition of weight maintenance (month 4 after weight loss and first training period, $n = 12$) with the month 10 measurement and the final month 16 measurement (training and weight gain) was performed with ANOVA repeated measures.
2. Effect of training and body weight changes. The effect of training in the obese group after a weight reduction period was examined using an exercise protocol in the respiration chamber with an absolute work load based on 40% \dot{W}_{\max} at baseline. The measurements at baseline (month 0) and on the second day of month 16 ($n = 9$) were compared with paired *t*-tests.
3. Macronutrient composition of the diet. The effect of diet supplied in the respiration chamber (Hi.F vs Red.F) was analysed by comparison of the RQ and FQ of the diets at different time points ($n = 6$ in each diet group) with the non-parametric Friedman test.
4. Training status. The obese group at 16 months of training was compared with the lean, well-trained group to find out whether the possible change in substrate utilization due to the training intervention in the obese subjects was closer to that of the lean, well-trained subjects. Differences were analysed with unpaired *t*-tests.

In the text, tables and figures, data are presented as mean \pm standard deviation (s.d.). Physical characteristics and energy intake data ($n = 12$) were analysed using ANOVA repeated measures.

Results

Physical characteristics

Physical characteristics of the well-trained group differed significantly in almost all physical parameters from those of the obese group (tested non-parametrically); subjects were only matched for age (Table 1). In both the obese and well-trained randomized subgroups, receiving a Hi.F ($n = 6$ and $n = 8$ respectively) or Red.F diet ($n = 6$ and $n = 7$, respectively), no differences in physical characteristics were found within each group (Table 1).

At the end of the study the obese group that received a Hi.F-diet in the respiration chamber had trained on average for 2.1 ± 2.3 h/week, which was similar for the Red.F-diet receiving group, 2.0 ± 2.1 h/week. The number of training hours a week of the obese subjects

Table 1 Physical characteristics of the obese and well-trained subjects during the intervention period^a

	Obese				Well-trained
	0	4	10	16	
Age (y)	36.3 (5.1)				36.2 (7.2)
BW (kg) ¹⁻⁶	94.6 (13.9)	82.2 (12.0)	86.7 (13.2)	89.6 (13.5)	72.2 (5.9)
BMI (kg.m ⁻²) ¹⁻⁶	30.8 (3.0)	26.7 (2.2)	28.2 (2.7)	29.2 (3.0)	22.3 (1.7)
W/H ratio ^{1,2,4-6}	0.95 (0.04)	0.88 (0.04)	0.91 (0.04)	0.95 (0.05)	0.84 (0.04)
Body fat (%) ^{1,2,4-6}	29.1 (3.5)	22.6 (3.4)	26.7 (3.9)	28.4 (4.4)	14.1 (2.8)
FFM (KG) ¹⁻³	66.9 (9.6)	63.5 (8.7)	63.3 (8.3)	64.0 (9.4)	62.0 (5.3)
\dot{W}_{\max} (W) ^{1-3,5}	249 (37)	299 (38)	286 (43)	284 (48)	348 (34)
$\dot{V}O_{2\max}$ (L/min) ¹⁻⁶	3.0 (0.5)	3.6 (0.6)	3.2 (0.6)	3.3 (0.6)	4.4 (0.5)

^aThe baseline physical characteristics (mean \pm s.d.) of the 12 obese subjects and of the 15 well-trained subjects. The data for the obese group is presented at the start of the study (0 months), and at 4, 10 and 16 months. Significant differences over time for the obese are presented: ¹month 0 vs month 4; ²month 0 vs month 10; ³month 0 vs month 16; ⁴month 4 vs month 10; ⁵month 4 vs month 16; ⁶month 10 vs month 16.

Table 2 Food intake during the 3 days prior to the respiration chamber experiment^a

	Obese				Well-trained
	0	4	10	16	
EI (MJ/d) ⁴	10.0 (3.0)	8.8 (2.2)	8.6 (2.2)	8.1 (2.7)	14.2 (3.0)
CHO (En%) ⁴	44.4 (6.4)	43.5 (5.6)	44.8 (5.7)	43.0 (6.3)	50.4 (5.1)
Fat (En%)	33.3 (5.6)	36.0 (7.0)	34.5 (6.9)	37.7 (7.7)	32.9 (3.9)
Protein (En%)	14.9 (3.0)	15.9 (2.1)	16.4 (3.0)	16.4 (3.3)	15.2 (1.3)
Alcohol (En%) ¹⁻⁴	8.7 (7.3)	4.6 (4.8)	4.4 (5.9)	2.8 (5.0)	2.0 (2.1)
Fibre (g/d) ⁴	16.8 (9.6)	15.8 (5.1)	16.0 (2.7)	14.6 (4.2)	31.0 (8.0)

^aThe data on food intake for the obese group is presented at the start of the study (0 months), and at 4, 10 and 16 months. Significant differences over time for the obese are presented: ¹month 0 vs month 4; ²month 0 vs month 10; ³month 0 vs month 16; ⁴difference between the obese (average intake) and the well-trained group.

Table 3 Energy and substrate balances for the obese and well-trained group^a

	High-fat diet (n = 6)					Reduced-fat diet (n = 6)			
	4 months	10 months	16 months	Well-trained (n = 8)		4 months	10 months	16 months	Well-trained (n = 7)
EI	15.1 \pm 1.2	15.0 \pm 1.2	15.0 \pm 0.9	15.1 \pm 1.2	EI	15.1 \pm 1.9	14.9 \pm 2.3	15.1 \pm 1.9	14.4 \pm 1.2
EE	15.2 \pm 1.2	15.0 \pm 1.2	15.1 \pm 1.1	14.7 \pm 0.8	EE	15.5 \pm 1.7	15.3 \pm 2.0	15.5 \pm 2.0	15.0 \pm 1.7
EB	-0.1 \pm 0.3	0.0 \pm 0.6	0.0 \pm 0.5	0.4 \pm 0.7	EB	-0.5 \pm 0.7	-0.5 \pm 1.2	-0.3 \pm 1.0	-0.6 \pm 0.7
CHO in ³	221.5 \pm 23.0	226.6 \pm 21.1	227.5 \pm 17.0	221.0 \pm 18.6	CHO in	462.9 \pm 57.8	460.3 \pm 67.2	471.0 \pm 57.4	431.5 \pm 24.0 ⁵
CHO ox ¹⁻³	239.4 \pm 52.3	325.0 \pm 105.7	315.3 \pm 23.1	406.4 \pm 27.7 ⁴	CHO ox	432.1 \pm 70.0	510.9 \pm 93.7	511.2 \pm 100.4	534.4 \pm 101.2
Δ CHO ^{1,2}	-17.9 \pm 41.8	-98.4 \pm 101.2	-87.8 \pm 26.2	-185.4 \pm 33.0 ⁴	Δ CHO	30.8 \pm 39.6	-50.5 \pm 114.9	-40.3 \pm 58.1	-103.6 \pm 97.1
FAT in ³	232.2 \pm 16.8	227.8 \pm 17.8	230.4 \pm 11.9	237.3 \pm 17.2	FAT in	117.0 \pm 15.9	115.0 \pm 19.7	116.4 \pm 15.4	120.5 \pm 14.5
FAT ox ¹⁻³	226.6 \pm 34.0	184.7 \pm 43.1	192.0 \pm 22.4	145.3 \pm 17.9 ⁴	FAT ox	142.6 \pm 9.7	104.5 \pm 44.6	107.7 \pm 33.4	90.5 \pm 43.2
Δ FAT ^{1,2}	5.6 \pm 24.8	43.0 \pm 47.7	38.4 \pm 12.7	92.0 \pm 10.3 ⁴	Δ FAT	-25.7 \pm 11.2	10.5 \pm 33.2	8.7 \pm 37.4	30.0 \pm 37.8
RQ ¹⁻³	0.80 \pm 0.02	0.83 \pm 0.03	0.83 \pm 0.01	0.86 \pm 0.01 ⁴	RQ	0.86 \pm 0.01	0.89 \pm 0.03	0.89 \pm 0.02	0.90 \pm 0.03 ⁵
FQ ³	0.79 \pm 0.00	0.79 \pm 0.00	0.79 \pm 0.00	0.79 \pm 0.00	FQ	0.87 \pm 0.00	0.87 \pm 0.00	0.87 \pm 0.00	0.87 \pm 0.01 ⁵

^aTwenty-four hour EI, RQ and FQ, and substrate balances (mean \pm s.d.) of the obese group when fed a Hi.F-diet and a Red.F-diet, at 4, 10 and 16 months, and of the well-trained group. The energy intake (EI), energy expenditure (EE) and energy balance (EB) are presented in MJ/d and the carbohydrate intake (CHO in), oxidation (CHO ox) and balance (Δ CHO), and fat intake (fat in), fat oxidation (fat ox) and balance (Δ fat) in g/d. Significant differences are indicated with ¹4 months vs 10 months; ²10 months vs 16 months; ³between diets (Hi.F vs Red.F diet); ⁴obese vs well-trained at Hi.F; ⁵obese vs well-trained at Red.F.

was significantly increased compared with the sedentary status at the beginning of the study. Of the well-trained subjects the Hi.F-diet group trained 13.3 ± 9.7 h/week and had a training history of 18.8 ± 8.9 y; the Red.F-diet group trained 10.9 ± 3.8 h/week and had a training history of 20.6 ± 6.7 y.

The recorded amount of food consumed *ad libitum* prior to the chamber experiments did not change very much over time (Table 2). However, clear differences in amount and macronutrient composition were found in diet consumed by the obese and by the well-trained group. Considering the differences in recorded EI and

measured EE, under-recording of approximately 40% must have occurred for the obese, but of less than 5% for the lean subjects.

Long-term training effect and the effect of body weight gain

Both the Hi.F diet and the Red.F diet group were in EB during the 24 h respiration chamber experiments performed at 4, 10 and 16 months (Table 3). The relative work load of 40% \dot{W}_{\max} at 4 months was 117 ± 12 and 122 ± 19 W for the Hi.F and Red.F diet group, resp. Six months later the 40% \dot{W}_{\max} was, on

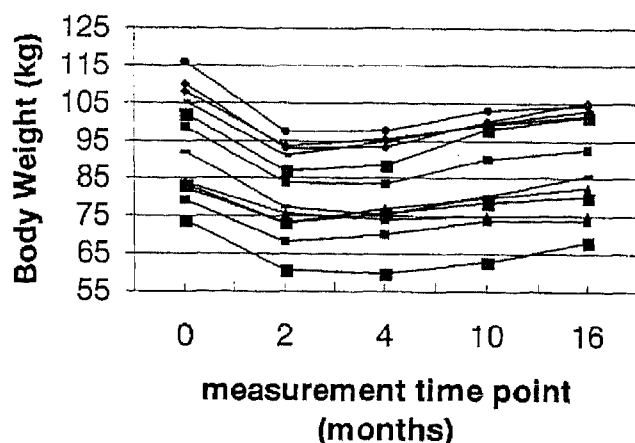


Figure 1 The BW changes of the 12 obese subjects presented at 0, 2, 4, 10 and 16 months.

average, 114 ± 15 W for the Hi.F diet group, and 115 ± 21 W for the Red.F group. At 16 months the work load cycled in the chamber was 113 ± 15 and 114 ± 24 W for the Hi.F and Red.F diet groups. The relative work load was not significantly different over time in either of the groups, nor did it differ between the diet groups.

Negative CHO balances were found for both diets, especially at 10 and 16 months. For both diets a difference of 70–80 g of CHO in comparison to the month 4 measurement was found. During the training intervention the fat balance became more positive (an increase of ± 35 g). Table 3 further illustrates that the substrate oxidation of the obese group at 10 and 16 months shifted towards the oxidation shown by the well-trained group.

Training and body weight changes

The effect of training, weight loss and weight gain were examined by comparison of 24 h energy and substrate balance at baseline (month 0) and at the second 24 h respiration chamber day at 16 months. The changes in BW are shown for all subjects individually (Figure 1).

The subjects exercised throughout the day at the same absolute work load (Hi.F group, $n=5$, 102 ± 6 W; Red.F group, $n=4$, 106 ± 17 W) determined at month 0 and a comparable, individual negative energy balance was imposed (-1.2 ± 1.1 MJ) at 16 as at 0 months. Despite the negative EB, the CHO and fat balances of both diets indicated that subjects relied mostly on CHO at 16 months. The results measured at month 0 and month 16 (second day) (Table 4) with a fixed work load are in accordance with the results presented in Table 3 for the chamber experiments with relative work loads (Δ (Hi.F vs Red.F): Δ CHO, -16.0 ± 36.0 vs -70.0 ± 25.8 g/d, $P < 0.05$; Δ fat, 6.4 ± 21.8 vs 34.9 ± 22.5 g/d, $P < 0.1$). The difference in CHO balances (Δ (Δ CHO)) at month 16 (second day) and the month 0 measurement of -54 g of CHO per 24 h indicates that, for the same protocol, more CHO was oxidized at month 16. The difference in fat balances (Δ (Δ fat)) ($+28$ g) also indicates that subjects oxidized even less fat than at the start of the study.

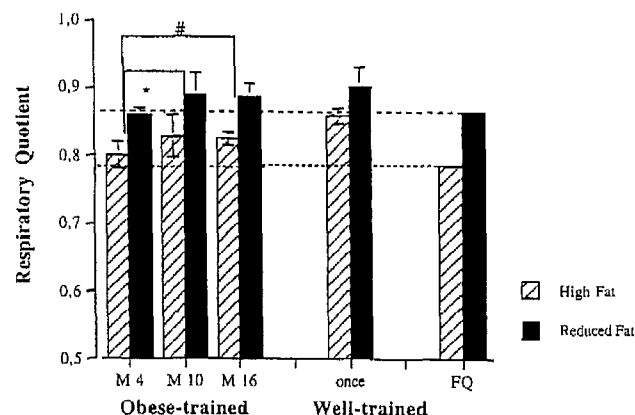


Figure 2 Twenty-four-hour respiratory quotients (RQ) and food quotient (FQ) (mean \pm s.d.) of the high-fat (hatched bars) and reduced-fat (filled bars) diet at 4 months (M 4), at 10 months (M 10), and at 16 months (M 16) for the obese group and of the once-measured well-trained group. * $P < 0.05$ between month 4 and month 10 (between both diets); # $P < 0.05$ between month 4 and month 16 (between both diets).

Table 4 Energy and substrate balances for the obese group at month 0 and month 16/day 2^a

	High-fat diet ($n=4$)			Reduced-fat diet ($n=5$)	
	0 months	16 months/day 2		0 months	16 months/day 2
EI	14.5 ± 0.9	14.4 ± 0.9	EI	14.7 ± 1.9	14.3 ± 1.7
EE	15.5 ± 1.1	15.5 ± 0.6	EE	16.2 ± 1.4	15.6 ± 2.3
EB	-1.0 ± 0.3	-1.0 ± 0.6	EB	-1.4 ± 1.5	-1.3 ± 1.4
CHO in	214.7 ± 11.3	220.9 ± 13.3	CHO in	448.0 ± 54.8	448.6 ± 54.4
CHO ox	238.9 ± 34.2	261.5 ± 29.9	CHO ox	422.0 ± 61.7	493.4 ± 71.7^1
Δ CHO	-24.2 ± 32.8	-40.6 ± 20.4	Δ CHO	26.0 ± 32.8	-44.8 ± 21.8^1
FAT in	223.5 ± 14.3	219.7 ± 14.2	FAT in	115.9 ± 17.1	109.1 ± 13.7^1
FAT ox	237.7 ± 28.6	227.6 ± 10.9	FAT ox	164.0 ± 27.9	122.3 ± 36.8^1
Δ FAT	-14.2 ± 18.2	-7.8 ± 20.7	Δ FAT	-48.1 ± 33.6	-13.2 ± 36.4^1
RQ	0.80 ± 0.01	0.80 ± 0.01	RQ ¹	0.85 ± 0.02	0.88 ± 0.02^1
FQ	0.79 ± 0.00	0.79 ± 0.00	FQ	0.87 ± 0.00	0.87 ± 0.00

^aEnergy and substrate balances (mean \pm s.d.) of the obese (OB) subjects when fed a Hi.F-diet or a Red.F-diet at 0 months and at 16 months/day 2. The energy intake (EI), energy expenditure (EE) and energy balance (EB) are presented in MJ/d and the carbohydrate intake (CHO in), oxidation (CHO ox) and balance (Δ CHO), and fat intake (fat in), fat oxidation (fat ox) and balance (Δ fat) in g/d. Significant differences between month 0 and month 16/day 2 are indicated with ¹.

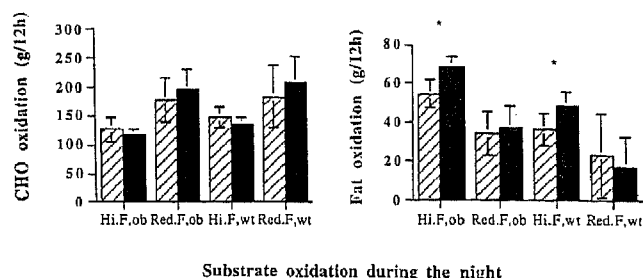


Figure 3 (a) The carbohydrate oxidation measured during the first and second night in the respiration chamber from 19.00 to 7.00 h. Carbohydrate oxidation (total over 12 h) is presented for the obese group (ob) at 16 months and the well-trained (wt) group for the high-fat (Hi.F) and reduced-fat (Red.F) diet during the first (hatched bars) and the second night (filled bars). No differences in CHO oxidation were found between the first and second night. (b) The fat oxidation measured during the first and second night in the respiration chamber from 19.00 to 7.00 h. Fat oxidation (total over 12 h) is presented for the obese group (ob) at 16 months and the well-trained (wt) group for the high-fat (Hi.F) and reduced-fat (Red.F) diet during the first (hatched bars) and the second night (filled bars). Significant differences in fat oxidation were found between the first and second night for the Hi.F-consuming groups (indicated with *).

The data presented in Table 4 show that no differences in substrate oxidation had taken place on a high-fat diet, but were clear on a reduced fat diet.

Macronutrient composition of the diet

The effect of the macronutrient composition of the diet on substrate oxidation is presented in Figure 2 for the obese group and the well-trained group. Comparison of the FQ of the diet and the measured RQ of the well-trained subjects showed significant differences. For both diets the 24 h RQ value, measured from 0.00 h to 0.00 h the next day, was higher than the FQ value at all time points for the obese group (range 0.01–0.04 for the Hi.F diet, significantly higher RQ at 10 and 16 months; range 0.00–0.02 for the Red.F diet, RQ not significantly different from FQ). These differences were even more pronounced in the well-trained group: a difference in RQ and FQ of 0.07 was found for the Hi.F diet, and a difference of 0.04 for the Red.F diet ($P < 0.05$). The obese group showed changes in RQ in the course of the study in the

direction of the well-trained group. However, the 24 h RQ values measured after 16 months of training were still significantly lower compared with the 24 h RQ values of the well-trained group, on the Hi.F diet (Hi.F diet: RQ obese 0.83 ± 0.01 vs well-trained 0.86 ± 0.01 , $P < 0.001$). For the Red.F diet the differences in RQ were not significant (Red.F diet: RQ obese 0.89 ± 0.02 vs well-trained 0.90 ± 0.03 , NS). These findings are further illustrated in Table 3.

No relation was found between the CHO–fat ratio of the diet prior to the chamber experiment (see Table 2) and the substrate oxidation measured in the chamber (for CHO intake and CHO oxidation, $r = 0.19$, NS; for fat intake and oxidation $r = 0.18$, NS).

Training status

To find out how the 24 h positive fat balances and negative CHO balances could arise, as found at month 16 (day 1) and especially for the lean, well-trained group (see Table 3), the data were further analysed by comparison of substrate oxidation during the first and second night (a 12 h measurement from 19.00 to 7.00 h). In Figure 3(a) the amount of CHO oxidation is presented for the Hi.F and Red.F diets in the obese group (at month 16) and the well-trained group for the first and second night in the respiration chamber.

No differences between the first and second night were found in CHO oxidation with ANOVA-repeated measurements. For fat oxidation, however, as presented in Figure 3(b), the differences between the first and second night were significant ($P < 0.05$). An increase in fat oxidation was found in the Hi.F-groups in the second night.

Because clear negative CHO balances were found for 24 h data (Tables 3 and 4) and no differences were seen in CHO oxidation of night 1 compared with night 2 (Figure 3(a)), a large amount of CHO had to be oxidized during the day. RQ of the different parts of the day were calculated, for resting metabolic rate (measured from 7.15 to 8.00 h), for 2 h cycling and the total 24 h RQ during the day. In Table 5 the different RQs are presented for the obese (at 4, 10 and 16 months) and for the well-trained group.

Table 5 Respiratory quotient in the respiration chamber^a

	Obese ($n = 6$)			Well-trained ($n = 8$)
	4	10	16	
High-fat diet				
RMR	0.81 (0.06)	0.80 (0.07)	0.81 (0.06) ³	0.93 (0.07)
Cycling (av.)	0.87 (0.02) ³	0.88 (0.05) ³	0.86 (0.02) ³	0.87 (0.03)
24 h	0.80 (0.02) ¹⁻⁴	0.83 (0.03) ³	0.83 (0.01) ³	0.86 (0.01) ³
Reduced-fat diet				
RMR	0.83 (0.04) ²	0.87 (0.03)	0.92 (0.06)	0.87 (0.13)
Cycling (av.)	0.92 (0.02)	0.94 (0.03)	0.91 (0.03)	0.89 (0.03)
24 h	0.86 (0.01) ⁴	0.89 (0.03)	0.89 (0.02)	0.90 (0.03)

^aThe respiratory quotient (RQ) is presented for the obese group at 4, 10 and 16 months, and for the well-trained group. The RQ is shown for the RMR, measured in the chamber from 7.15 to 8.00 h (under fasting conditions), averaged for the 2 h cycling, and over 24 h. Differences ($P < 0.05$) over time for the obese group between months 4, 10 and 16 are indicated with ¹month 4 vs month 10; ²month 4 vs month 16; ³significant differences between diets; and ⁴differences between obese and well-trained group.

Significant differences over time, between diets, and between the groups (as a consequence of the training status) were found for the 24 h RQ. The well-trained subjects showed significantly elevated RQs in comparison to the obese subjects for the HI.F-diet group as well as for the Red.F-diet group. During cycling the RQs differed significantly between diets, but not as a consequence of training status. All RQs measured differed significantly between the diets in the obese group, except for the RMR RQ at 4 months.

Regression analysis to clarify the factors related to CHO and fat oxidation (during the first and second night), were performed. Body fat percentage and hours of training weekly were used as factors to explain the variance in substrate oxidation in the first and second night. The relation between body fat% and fat oxidation increased in the second night ($r = 0.33$, $P = 0.09$) during the first night and $r = 0.41$, $P < 0.05$ during the second night). The changes in body fat% and fat oxidation were not at all related. Also, hours of training per week was not related to fat oxidation. No relation was found between body fat percentage or hours of training with CHO oxidation.

Discussion

Substrate oxidation

The data presented clearly indicate that the training intervention resulted in a higher reliance on CHO as the 24 h energy substrate in the course of the study, assuming a constant food intake and CHO–fat ratio. The results are supported by similar findings in a well-trained group, which indicate that life-long training results in increased 24 h CHO oxidation and less fat oxidation during a 24 h energy balance protocol in the respiration chamber with 2 h of moderate exercise. With respect to the substrate oxidation results found in the present study, two important factors should be stressed. Firstly, the results are collected under strict energy balance (except for the month 0 and month 16/second day). Secondly, the results presented are based upon 24 h data and include not only exercise performance data.

Our results are in line with a recently published study of Roy and coworkers.³² Trained and non-trained subjects also showed similar substrate oxidation in a respiration chamber, when consuming iso-energetic high- and low-fat diets. The type of diet that was consumed had a major effect at substrate oxidation, whereas prior athletic training (training history) had no effect on substrate oxidation,³² a result in agreement with our findings.

An explanation for the clear negative CHO balances (increased CHO oxidation) found could be the increase in absolute exercise performance (relative 40% \dot{W}_{\max}). The increased energy expenditure has to be generated by more fuel, and could therefore

explain, when an impaired fat oxidation is present, the increase in CHO oxidation. However, the results comparing the absolute work load at month 0 and month 16/day 2 (see Results section) showed a similar shift in substrate oxidation towards CHO. The work load cycled at the first 24 h respiration chamber day at month 16 was significantly higher (15%) in comparison to the baseline 40% \dot{W}_{\max} work load. This illustrates that it was worthwhile differentiating between absolute and relative work load. The oxidation of substrates, however, did not differ significantly, and stressed again that the 16 months exercise intervention did not improve fat oxidation in the obese subjects.

We are aware of only two other studies reporting an increase in CHO oxidation after an exercise intervention. In the first study post-obese women trained two to three times a week for 45–60 min outdoor running and cycling for 3–4 months.³³ Although the intensity and frequency of the training sessions was lower in comparison to the present study, on average a comparison with our study is very well possible. Sleeping and 24 h RQ were found to be significantly increased after the training intervention, and an increased CHO availability after training was suggested.³³ Also Westerterp and coworkers reported an increase in RQ during the night.³⁴ Untrained, normal-weight male and female subjects participated in an endurance training intervention of 44 weeks, to complete a half-marathon. Overnight a change to relatively less fat and more CHO oxidation was found in the course of the training intervention.³⁴ Their suggestion that training could result in an increased insulin sensitivity, and therefore end up with higher glycogen stores, agrees with the suggestion of Tremblay and Buemann that more CHO was available stored as glycogen.¹¹ Increased glycogen levels in trained (584 mmol/kg dry weight) compared with untrained subjects (433 mmol/kg dry weight) have been found.¹⁶ Higher glycogen levels therefore might result in an increased 24 h CHO oxidation. Although the measurements in the present study were carried out under the condition of energy balance, we would like to suggest, besides the increase in insulin sensitivity and consequently higher CHO oxidation, that the increased CHO oxidation could be explained by lipogenesis or fat storage in the muscle. Predominant depletion of intramuscular triglycerides (IMTG) during exercise has been found in trained subjects, suggesting that the greater utilization of fat during exercise is due to increased lipolysis of IMTG.³⁵ These stores need to be replenished, which could possibly be done via increased 24 h CHO oxidation. Lipogenesis might therefore explain the increased 24 h CHO oxidation found with training.

We cannot exclude the possibility that the increased reliance on CHO as a substrate found may be a measurement error, since we did not measure nitrogen excretion to calculate protein balance. However, in our laboratory it was found repeatedly that protein intake balanced protein oxidation.^{25,27,28} Furthermore,

the finding that the well-trained subjects showed an even more pronounced 24 h CHO oxidation might suggest that other regulatory mechanisms are involved when substrate oxidation is examined for 24 h, instead of during short exercise bouts.

The 24 h negative CHO balances and positive fat balances found in the present study are in contrast to the general consensus that endurance training stimulates fat oxidation during exercise^{12,14–16} and during rest.^{9,13} The positive fat balances, even in the Red.F conditions, might indicate that the amount of fat supplied was too high or that fat oxidation was reduced. During the training intervention the fat balance became even more positive (an increase of +35 g), already indicating that fat oxidation did not improve. The similar differences found in the CHO balance (70–80 g) and the fat balance (± 35 g) for both types of diets, indicate that a 1 y training program did not alter metabolic capacity to increase fat oxidation. An increased CHO oxidation via increased insulin sensitivity and possibly muscle lipogenesis might explain decreased instead of increased fat oxidation.

Our findings are in contrast with previous findings in our laboratory. Van Dale and Saris³⁶ found in obese women participating in a diet and exercise program for 14 weeks, that fat oxidation had increased as a consequence of training. However, this was observed under a strong negative energy balance, which is also the case in the present study from month 0 to month 4. In the present study the fat oxidation was also highest at 4 months; substrate oxidation matched substrate intake, even with a Hi.F diet. This is especially clear from the RMR-RQ data at 4 months (Table 5). The training effect was achieved after 4 months and training status did not increase, as already discussed elsewhere.³⁷ However, maintenance of the physical fitness was possible, although BW gradually increased in the last 12 months.

Macronutrient composition of the diet

Fat oxidation was relatively higher in the groups that received a Hi.F-diet compared with the groups that received a Red.F-diet. Increased fat oxidation when Hi.F diets are supplied has been found before.^{28,38} This finding is in contrast with the result of Schutz, who observed no increased fat oxidation after addition of 106 g of fat to a meal.⁵ The equicaloric exchange in macronutrients as carried out in our study might explain this difference. Because less CHO was consumed with the Hi.F diet, less CHO oxidation took place and fat oxidation will increase on a Hi.F diet.³⁰ A possible explanation for the fast adaptation of substrate oxidation to substrate intake could be the increased energy turnover due to the exercise bouts, as was already shown by Schrauwen and colleagues.³⁹ The effect of fat intake at fat oxidation is assumed to be similar at all measured time points. The increased positive fat balances found at 16 months, under

similar testing conditions, shows independently of the fact that fat is added or CHO replaced by fat, that less fat was oxidized in the course of the study.

In the present study the Hi.F or Red.F diet given in the respiration chamber was supplied to the subjects acutely. The diet consumed in the chamber was not supplied the days before at home, so no adaptation to the diet could take place. Based upon the reported food intake it is unlikely that the type of food consumed prior to the respiration chamber measurement affected substrate oxidation measured in the chamber. However, food intake was clearly under-reported when compared to EE. Therefore we cannot draw firm conclusions in this respect. A carry-over effect of prior diet consumption could cause the actual substrate oxidation in the respiration chamber. On the other hand, there are no indications that this inconsistency on an individual base will change due to this type of intervention. The differences in oxidation response found after macronutrient intake (Figure 3) indicate that CHO oxidation was already similar to intake during the first night, stressing a fast adaptation. This was not found for fat balances; adaptation to a fat balance has recently been found to be more time consuming (one week),³⁹ but might be shortened by the increased energy turnover in our subjects.

The changes in substrate oxidation caused by training should preferably be studied with the data of the first measurement at month 0, and compared with month 16 (day 1). However, because of the clear negative energy balance at the start, as a result of a higher actual EE than estimated EE and supplied EI, we decided to study the effect of training when subjects were in energy balance after weight loss (at month 4) and compare the results with the results obtained at month 16 (day 1). The second day of the month 16 measurement was carried out to obtain the same negative energy balance and also the same absolute work load, to create an identical situation in order to study the effect of training over the complete 16 month period. These results confirmed the finding that endurance training resulted in increased 24 h CHO oxidation.

Conclusions

Endurance training did not result in an increased fat oxidation over 24 h, as is often found for the training performance itself. Data from well-trained subjects supported the finding that during this 24 h energy balance protocol in the respiration chamber mostly CHO was oxidized. It was further found that the acute change in CHO–fat ratio in the diet supplied in the chamber affected the substrate oxidized, resulting in relatively higher fat oxidation on a high-fat/low-carbohydrate diet, and relatively higher carbohydrate oxidation on a low-fat/high-carbohydrate diet. Future

studies should analyse the discrepancy of substrate oxidation during exercise and 24 h substrate oxidation.

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